The Hybridization of Aedes aegypti and Aedes albopictus in Hawaii

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In the Territory of Hawaii, there are three species of mosquitoes: *Aedes albopictus* (Skuse), *Aedes aegypti* (Linn.), and *Culex quinquefasciatus* Say. Usinger (1944) has reviewed the biology of the two *Aedes* species in connection with an epidemic of dengue which occurred in Honolulu in 1943-44. Mention is made of various experiments in which the hybridization of these species had been tried. From time to time various authors have reported attempts to cross different species of mosquitoes. In only a few of these attempts has success been reported. Weyer (1936) and Roubaud (1941) successfully crossed *Culex pipiens* with *Culex quinquefasciatus* in Europe. Various workers have reported hybridization experiments in species and varieties of anopheline mosquitoes (cf. Corradetti, 1934, 1937A, 1937B; de Buck, *et al.*, 1934, 1937; Roubaud, *et al.*, 1936, 1937; Diemer and Van Thiel, 1936; Sweet, *et al.*, 1938; Bates, 1939). Toumanoff (1937, 1938, 1939) reported successful crossing in Indo-China between *Aedes aegypti* and *Aedes albopictus* while cross-breeding experiments with material from Calcutta and Indo-China were unsuccessful (Toumanoff, 1937). Simmons, St. John and Reynolds (1930) were unsuccessful in crossing these species using material from the Philippines. Hoang-Tich-Try (1939) reported that male *A. aegypti* and female *A. albopictus* crossed readily in small cages and at unfavorable times of the year. In this report of successful crossing all the progeny resemble *A. albopictus* except in a single specimen. De Buck (1942) reports that he was unsuccessful in hybridizing laboratory strains of *A. aegypti* and *A. albopictus,* but microscopic examination of the spermatheca in the female of both species demonstrated the presence of spermatozoa. No embryonic development occurred in eggs laid by female *A. albopictus* and the few larvae that hatched from eggs laid by *A. aegypti* died in several hours. Downs and Baker (1949) reported success in crossing female *A. aegypti* and male *albopictus,* while the reciprocal cross failed. MacGilchrist (1913) observed in Calcutta "that individuals of the species *fasciatus* [*A. aegypti*] not infrequently copulated with individuals of the specie *scutellaris* [*A. albopictus*]; but this coupling seems to be unproductive." Connal (1925) attempted to cross-breed *A. aegypti* with *Aedes luteocephalus* and *Aedes longipalpus* in Africa but with negative results.

In the course of the mosquito control program sponsored by the Territorial Board of Health, the Honolulu Chamber of Commerce and the U. S. Public Health Service, a small laboratory was established. In addition to the regular work of the laboratory directly connected with

control activities, there was an opportunity to perform a few experiments on the hybridization of *A. aegypti* and *A. albopictus*.[1]

METHODS

Adult mosquitoes for this experiment were obtained by removing pupae from laboratory cultures of *A. aegypti* and *A. albopictus*. Each pupa was segregated in an individual cotton-stoppered vial. After the emergence of the mosquitoes, each was individually examined and separated into groups according to sex and species. Extreme care was observed and duplicate observations by two workers were made to insure accuracy. Male *A. albopictus* and female *A. aegypti* were introduced into one cage and male *A. aegypti* and female *A. albopictus* were introduced into another cage. These cages were supplied with moist raisins, sugar water and a growing plant (*Philodendron* sp.) as food for the mosquitoes. These cages were placed in a screened room and were each enclosed in a mosquito bar as an additional safeguard. From time to time at irregular intervals, human blood meals were provided and the vials examined for eggs. Two trials with male *A. aegypti* and female *A. albopictus* were unsuccessful since no fertile eggs were obtained. In the reciprocal cross with female *A. aegypti* and male *A. albopictus*, two out of three trials produced fertile eggs and living larvae. The larvae were removed and placed in covered containers and fed upon rice or bread crumbs. As the adults of the F_1 generation emerged, they were collected and examined to determine sex and species. In several cases the fourth instar larva or larval skins were mounted for microscopic examination. The male terminalia of some of the F_1 males were prepared and mounted on slides for comparison with terminalia of males of the two local *Aedes* species. In one experiment, the F_1 generation was placed in a cage and allowed to inter-breed and the offspring were also examined. This process of in-breeding was repeated for five generations as follows:

9/10/44—4 female *A. aegypti* and 8 male *A. albopictus* introduced into cage.
9/11/44—2 females took a blood meal.
9/14/44—1 female took a blood meal.
9/18/44—Larvae and eggs observed in a glass jar and a glass vial. Larvae removed to cloth-covered container and provided with rice.
9/30/44—1 male mosquito emerged.
10/ 3/44—12 adults emerged by this date, 4 females and 8 males.
10/ 6/44—4 females and 6 males emerged.
10/ 7/44—All specimens of the F_1 generation placed in a large breeding cage.
10/ 7/44—3 F_1 females took a blood meal.
10/ 9/44—First instar larvae present in the F_1 breeding cage.
10/17/44—First F_2 adults emerged.

[1] These experiments were, unknowingly and simultaneously, carried on at the same time that similar work was being done at the Rockefeller Foundation by Dr. Wilbur Downs and Rollin Baker. The results are mutually confirmatory which adds significance to these experiments.

10/17/44
to —Between these dates 182 males and 63 females had
11/12/44 emerged.
10/23/44—A group of F_2 males and females were placed in a breeding cage.
11/10/44—46 males and 30 females of the F_3 generation had emerged.
11/13/44—10 males and 6 females of the F_3 generation were placed in a breeding cage.
11/25/44—18 males and 6 females produced in the F_4 generation.
12/20/44—3 males and 4 females secured in the F_5 generation.

Examination of all specimens obtained in this experiment has shown them to appear exactly like *A. aegypti.* There was no evidence of any modification of morphological structure that could be ascribed to the male *A. albopictus* parent. Examination of the terminalia of males in each of the several generations showed them to be identical with the terminalia of pure *A. aegypti* males. Examination of the larvae and pupae did not show any difference from normal *A. aegypti* larvae that could be detected. From the results of this experiment it can be concluded that fertile eggs can be produced from matings of female *A. aegypti* and male *A. albopictus.* The offspring resembled the maternal parent and showed no external morphological evidence of a paternal contribution. Unless the mechanism of sex determination is different from the condition found in other Diptera, parthenogenesis cannot account for these results since both sexes were obtained and they were fertile amongst themselves.

In order to determine the presence of a paternal contribution, the following back-cross experiment was performed with the females of the F_1 hybrid generation obtained in a hybridization experiment similar to that which has been described above. The F_1 females, which all had the markings of *A. aegypti,* were placed in a breeding cage with a large number of carefully identified and segregated male *A. albopictus.* A summary of the experiment is presented here:

12/21/44—5 female F_1 hybrids, resembling *A. aegypti,* were placed in a breeding cage with 22 selected male *A. albopictus.*
12/22/44—1 female took a blood meal. No copulation observed although several attempted copulations were noted.
12/27/44—An additional 20 male *A. albopictus* placed in the cage. Many attempted copulations but of short duration.
12/29/44—1 vial containing approximately 35 eggs removed from cage.
1/ 2/45—Many successful copulations observed. Two batches of eggs totaling 80 in vials removed from cage.
1/ 5/45—1 larva hatched from eggs removed on 1/2/45. Females took blood meal. Many successful copulations observed prior to and after blood meal.
1/12/45—Larva successfully pupated. Fourth instar skin mounted.
1/13/45—Adult male mosquito emerged from pupa. Appears as *A. albopictus.* 20 additional eggs removed from cage in vials.
1/21/45—2 female F_1 mosquitoes remained. 1 took a blood meal. 25 additional *A. albopictus* males added. 40 eggs removed from cage.

1/23/45—From the total of 175 eggs, only a single larva already mentioned was obtained. Attempts to induce hatching of the other eggs were unsuccessful.
1/26/45—No additional eggs obtained. Experiment discontinued.

An examination of the single specimen produced by back-crossing of females of the F_1 hybrid generation (*A. aegypti* × *A. albopictus*) showed the specimen to be indistinguishable from normal *A. albopictus* males. The fourth instar larval skin was identical in appearance to the normal larval skin of *A. albopictus*. An examination of the terminalia of this single male showed it to be exactly similar to the terminalia of a pure *A. albopictus* male. This specimen developed from eggs laid in a clean vial and there is no doubt but that these eggs were laid by the female hybrids. There can be no doubt concerning a true hybridization in the formation of the F_1 generation. Unfortunately neither equipment nor time was available to study the chromosome pictures in these specimens produced by these hybridization experiments. (cf. Sutton, 1942.)

From the control standpoint, it is of considerable interest that female *A. aegypti* can mate with male *A. albopictus* and produce fertile eggs. It is not known whether this can occur outside of the laboratory. There is, however, a possibility that in an area where the ranges of these mosquitoes overlap, such as in Hawaii or Asia, such an occurrence would possibly complicate aspects of control.

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